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(54) Title: HUMAN NUCLEIC ACID FRAGMENTS, ISOLATED FROM BRAIN ADRENAL TISSUE, PLACENTA OR BONE NARROW

(57) Abstract

This invention provides a nucleic acid fragment encoding a gene product or portion thereof and comprising any one of: (a) a sequence selected from SEQ ID Nos 1 to 1193 from the attached sequence listings; (b) an allelic variation of a sequence as defined in (a); or (c) a sequence complementary to (a) or (b). The invention includes uses of such fragments, and gene products corresponding thereto.

D. M. et al., p108 Blackwell scientific Publications, Oxford. Harlow, E and Lane, D. Antibodies, A Laboratory Manual, Cold spring harbor Laboratory, Cold Spring, New York.

Expression in an appropriate higher eucaryotic host may be important to ensure correct protein folding and also activity. Expression to avoid copurification of toxic products can sometimes be better performed in organisms approved for human consumption, eg prokaryotic Bacillus subtilis, eurkaryotic yeast, mammalian cows milk vectors, and other methods known in the art.

The invention also includes novel gene products or portions thereof encoded by a fragment, sequence or gene-comprising DNA fragment of the invention.

It will be appreciated that the sequences of the present invention collectively have utility based, inter alia, upon their common origin, and hence they can effectively be considered together rather than as separate entities. It is convenient to represent them as separate sequences, because this is how they were produced and serves as "punctuation" between the different functional entities which each sequence represents. However, the sequences could just as easily have been presented as a continuous sequence derived by placing them end to end in the order in which they were produced, with a separate indication of where the beginnings and ends of the component sequences are.

In contrast to investigations hitherto, where gene fragments (sequence fragments) could only be identified through some known characteristic (for example: their homology to a fragment which largely encodes amino acids identified by sequencing a previously isolated peptide or is the antisense of that coding sequence; or them having at least partial homology to previously characterised nucleic acids; or them having ability to encode expressed proteins which could later be detected by functional assays of the cells expressing those proteins or by using antibodies which had been previously raised against the proteins to detect their expression, Sambrook J., et al., Molecular Cloning CSH Press 1989), the sequences and fragments described by the present invention are entirely underivable and unpredictable from the prior art, but are nonetheless clearly of great value for various purposes.

Thus, such sequences, by comparing them to sequence databases, can be

used as a means for determining the existence of new members of existing gene families, new human genes when previously only non-human genes were known and new genes when previously no genes were known (Karlin, S. and Altschul, S. F. Proc. Natl. Acad. Sci. 87 p2264-2268 (1980)). In all cases, this allows the isolation of the corresponding genes and their products, and hence enables the manufacture of molecules of potential biological interest by recombinant means. Screening libraries of known materials or hitherto unexplored source materials for biological efficacy is now an important industrial activity in the search for new therapies and therapeutics. When new sequences have already been found to have counterparts in gene families or in non-human genes then knowledge about biological efficacy may already be apparant. For example, new receptors or receptor agonists/antagonists may exhibit differences to known instances of these molecules, and such differences could make them more suitable as therapeutics by, for example, exhibiting binding characteristics which are more in keeping with avoidance of toxicity. Reference can be made, for example, to polymorphic dopamine receptors and the implications for mental health (Iversen, L. Nature 358, p109 (1992), and Van Tol, H. M. M. et al., Nature 358, p149-152 (1992)). Where absolutely required, realisation of full length cDNAs for expression can be achieved by using the sequences to screen (by hybridisation) suitable cDNA libraries containing full length clones (D'Alession, J. M., et al., Focus (Gibco B.R.L) 9 p1 (1987)). Alternatively, the sequences can be used to design primers suitable for obtaining the missing sequences by PCR or other amplification methods (Frohman, M. A., Dush, M. K. and Martin, G. R., Proc. Natl. Acad. Sci. 85 p8998-9002 (1988)).

Appropriate use of the sequence fragments in antisense or triple helix (Griffin et al., Science 245 p967-971 (1989)) applications will be useful for identifying manipulable targets related to disease. For example, viruses have been inhibited by antisense RNA to their mRNAs (Chang, L-J., and Stoltfuz, C. M. J. Virol. p921-974 (1987)). A similar effect could be achieved by targetting the expression of cellular proteins which are essential for growth or maintenance of the virus.

Partial or full length cDNAs have great utility once expressed. The manner of expression can be selected by one skilled in the art to suit the intended application. Expression of full length cDNAs is typically required for biological activity. Procaryotic, and lower or higher eucaryotic hosts may be selected as the host for expression and higher

eucaryotes may be preferred to ensure correct modifications, for example, glycosylation in vivo, when this proves to be important. Expression can be ensured by situating the cDNA appropriately to signals for expression (Amann, E. and Brosius, J. Gene 40 p183 1985), Shimuzu, Y et al., Gene 65, p141 (1988), Straus, D. and Gilbert, W. Proc. Natl. Acad. Sci. 82, p2014 (1985)). Such signals may include a promoter for transcription, which may itself be regulatable.

The proteins thus-expressed can be screened for activities of therapeutic or commercial value. It may be that the proteins have to be first isolated for this purpose or can be assayed in situ. It may be desirable that some means of stabilising the expressed protein is employed. This can be achieved, for example, (and as indicated earlier) by expressing in frame as part of a fusion polypeptide (Smith, D. B., et al., Proc. Natl. Acad. Sci. 83 p8073 (1986)).

Useful antibodies can be raised against the expressed proteins. It is commonly not an absolute requirement that full length proteins are produced, although this may influence the quality of the antibodies produced. Peptides as short as 8 or 9 amino-acids in length can be used as antigens (Germain R., N. Nature 353 pp605-607 (1991), Rudensky, A., Y., et al., Nature 353 p622-627 (1991)). Immunogenic peptides could simply be synthesised using the amino-acid sequence translated from a sequence or fragment of this invention. It is desirable, although not absolutely required, that some means of producing purified antibodies is adopted. When fusion polypeptides are used to raise antibodies, an affinity matrix specific for the generic part of the protein allows the fusion polypeptide to be immobilised (Smith, D. B., et al., Proc. Natl. Acad. Sci. 83 p8073 (1986)). The immobilised polypeptide can then be used to affinity purify the antibodies. Antibodies to both the generic part of the fusion polypeptide and the part of interest are produced. When these need to be discriminated between, a different affinity column can be used to remove only those antibodies specific for the generic part of the polypeptide. Alternatively, and as mentioned earlier, it can be arranged that the boundary between the two separate protein components of the fusion polypeptide has the recognition sequence for an endopeptidase with a rare cutting site. The peptide of interest can then be released from the affinity purified polypeptide by the action of the endopeptidase (Nagai, K., and Thogersen, H., C. Methods Enzmol. 153 p461-481 (1987). Another alternative is raise monoclonal antibodies against the purified protein.

The antibodies can be used for localising in situ, or quantifying in samples through, for example, ELISA or RIA assays, peptides against which they were raised. These uses are particularly beneficial when the results of the assays can be correlated to a disease condition, eg cancer. For example tumour markers may be found and used to target therapeutic agents. The antibodies can also be used to detect or monitor markers of undifferentiated growth, infection, cardiovascular or immune disease or a therapeutic response. When the antibodies recognise cell surface proteins they can be used in isolation or in combination to isolate particular populations of cells. These in turn can be used to isolate yet more cDNAs which will be enriched for yet more of such surface markers for the population, which, if similarly screened, will permit yet further subdivision of the population. Ultimately, panels of antibodies which can describe particular disease states will accrue. Such antibodies could be tailored for forensic applications as well as diagnostic purposes and disease monitoring.

The sequences or fragments can also be used for genetic analysis and mapping, for example, to diagnose the likelihood that a given individual is predisposed towards a given genetic disease. In the event of a sequence co-locating, genetically, with a disease gene, it can be used for the derivation of new disease therapies bases upon precise genetic knowledge. Such therapies can include, for example, the techniques of so-called "gene therapy" (Dusty Miller, A. Nature 357 p455-460 (1992)).

Antibodies can be produced against the protein of a genetic disease with sufficient discriminating power to discriminate between diseased and non-diseased states (Caskey, T. Genome Sequencing Conference, Hilton Head, S. Carolina (1991)). This would be useful for reducing the dependence of such tests on nucleic acid-based screens. Such antibodies also have the advantage of allowing detection of faulty expression of the protein, for example levels of expression which may be important for development of the disease in slow onset conditions.

Also very important is that not all cDNAs are likely to be found by conventional means, whereas the present sequences are, in one sense, "comprehensive". The use of the class of cDNAs which corresponds of necessity to truncated clones increases the chances that part of a cDNA will be cloned free of any sequences that could otherwise compromise it from being cloned. Sequence obtained can then be used to generate PCR primers from which the remainder can be obtained without having to

clone.

This invention will now be further described and illustrated by means of the following Examples.

All oligonucleotides used in these Examples were synthesised Trityl on using an ABI 380B DNA Synthesizer according to the manufacturers instructions. Purification was by reverse phase HPLC (see, for example, Becker, C., R., et al., J. Chromatography 326, p293-299 (1985)).

Example 1

Human brain and adrenal tissues were obtained from a mixture of 12 to 15 week menstrual age foetuses and then snap frozen in liquid nitrogen before storing in bijou bottles in a -80°C freezer. The two types of tissue were used separately, directly from the freezer, to prepare cDNA from which restriction fragments were generated for sorting into subsets. 1g portions of each of the separate tissues were homogenised, using an Ultra-Turrax T25 Disperser (Janke and Kunkel, IKA-Labortechnik), on ice in the presence of 4M guanidinium isothiocyanate to solubilise macromolecules. RNA was isolated from each homogenate by using centrifugation to sediment it through caesium trifluoroacetate. This was performed using the Pharmacia kit according to the manufacturer's instructions, except that centrifugation was performed for 36 hours and the RNA obtained was finally desalted and concentrated by performing two ethanol precipitations in succession with two 70% ethanol washes after each precipitation. In each case, polyA$^+$ (mRNA) was isolated from 200 to 400 μg of the total RNA by binding it to magnetic oligo-dT coated beads (Dynal). Solution containing unbound material was removed from the beads, which were washed, and then mRNA eluted directly for use. mRNA isolation was performed in accordance with the manufacturer's instructions. Yields of RNA from the beads were between 1 and 3% of the total RNA. 2 to 4 μg of the eluted RNA were used for cDNA synthesis. cDNA synthesis was performed according to the method of Gubler, U and Hoffman, (B. J. Gene 25 p263 (1983) using a Pharmacia kit according to the manufacturer's instructions. OligodT was used to prime the first strand cDNA synthesis reaction. The cDNA was purified by extracting twice with phenol/chloroform and then low molecular weight solutes including nucleic acids below ca. 300 bases were removed by passing the cDNA reaction mixture through a Pharmacia S400 spun column used according to the manufacturer's